Technical Manual

Bode PunchPrep™

Instructions For Use Of Product P09D79

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Bode PunchPrep™ Kit

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1. Description

Bode PunchPrep™ is designed for processing punches from Bode Buccal DNA Collectors™ prior to the amplification with both Identifiler Direct ® and PowerPlex® systems for human STR genotyping. The Bode PunchPrep™ Kit contains twelve (n=12) single use vials sufficient for processing approximately three thousand (n=3000) samples*.

*Three thousand (n=3000) is the maximum number of samples that can be processed if the reagent is completely consumed. This does not factor in any additional volume of Bode PunchPrep™ needed to account for pipetting error. **All vials are single-use and should be discarded after first usage.**

Table 1. Direct Amplification Systems Compatible with Bode PunchPrep[1]™

AmpFlSTR® Identifiler® Direct
PowerPlex® 16HS
PowerPlex® 18D
PowerPlex® 21

[1] Other amplification systems may be compatible but are untested. Additional reagents may be required for systems not originally designed for direct amplification.

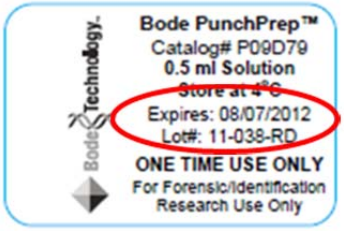
2. Components and Storage Conditions

Product	Part Number	Quantity
Bode PunchPrep ™ Kit	P09D79	12 X 0.5mL – One Time Use Bode PunchPrep ™ Vials

Not for Medical Diagnostic Use. For Forensic Identification/Research Use Only.

Storage Conditions: Bode PunchPrep™ is shipped at room temperature. Upon arrival, Bode PunchPrep™ should be stored at 4˚C. After removal from storage at 4˚C, the reagent should be mixed by vortexing and centrifuged briefly. Do not store Bode PunchPrep™ in the refrigerator door as the temperature may fluctuate. Reagent performance may be compromised by storing in the refrigerator door.

Expiration: Each vial is clearly marked with an expiration date. User should **always** check the expiration prior to use. If the product has passed its expiration date, a different lot of Bode PunchPrep™ should be used.



3. Suggested Procedures for Processing 1.2mm Punches from Bode Buccal DNA Collectors™.

Note: An initial sensitivity experiment must be performed with each desired amplification system to determine the optimal cycle. Cycle recommendations listed below were the optimal cycles determined during internal testing. Results may vary based on individual amplification and detection instruments.

a. AmpFlSTR® Identifiler® Direct

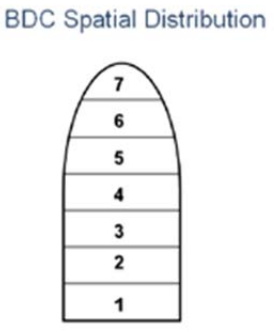
Materials to be Supplied by the User:

- 96 well reaction plate
- 70˚C heat block capable of accepting a 96 well reaction plate
- AmpFlSTR® Identifiler® Direct Amplification Kit

1. Place 96 well reaction plate under an ionizing fan for 5-10 minutes to eliminate static.

2. Punch one 1.2 mm punch from each Bode Buccal DNA Collector™ being processed into individual wells of the 96 well reaction plate.

Note: Remove the 1.2mm punch from an area close to the tip of the Bode Buccal DNA Collector™. Previous studies have shown that the top third of the Buccal DNA Collector™ contains the highest concentration of cells.



Position #	Position location on BDC
7	Tip
6	Near tip
5	Upper middle
4	Middle
3	Lower middle
2	Near base
1	Base

3. Apply 2µl of Bode PunchPrep™ directly onto the 1.2 mm punch in each well.

Note: Ensure that each 1.2mm punch is fully submerged in Bode PunchPrep™. **DO NOT** cover the plate with strip caps or another sealing material. **DO NOT** use a thermal cycler with a closed lid as this will prevent evaporation.

4. After applying the Bode PunchPrep™ solution to each sample, place the 96 well reaction plate on a heat block preset at a temperature of 70˚C. Keep the reaction plate on the heat block for twenty (20) minutes.

Note: Altering the incubation temperature and/or time may result in poor performance.

5. After the 20 minute incubation, remove the plate from the heat block.

6. Prepare the PCR amplification mix according to the AmpFlSTR® Identifiler® Direct Technical Manual.

7. Add 25µl of the AmpFlSTR® Identifiler® Direct amplification mix to each well.

8. Seal reaction plate with caps or an appropriate sealing material.

9. Centrifuge the reaction plate for 20 seconds at 3000 rpm.

10. Place the plate on a thermal cycler programmed according to the AmpFlSTR® Identifiler Direct® Technical Manual for 26 cycles.

11. When the amplification program has finished, continue processing the samples through the DNA analysis process per the user's specified processes.

b. PowerPlex® 16HS

Note: An initial sensitivity experiment must be performed with each desired amplification system to determine the optimal cycle. Cycle recommendations listed below were the optimal cycles determined during internal testing. Results may vary based on individual amplification and detection instruments.

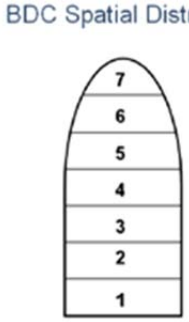
Materials to be Supplied by the User:

- 96 well reaction plate
- 70˚C heat block capable of accepting a 96 well reaction plate
- PowerPlex® 16HS System

1. Place 96 well reaction plate under an ionizing fan for 5-10 minutes to eliminate static.

2. Punch one 1.2 mm punch from each Bode Buccal DNA Collector™ being processed into individual wells of the 96 well reaction plate.

Note: Remove the 1.2mm punch from an area close to the tip of the Bode Buccal DNA Collector™.
Previous studies have shown that the top third of the Buccal DNA Collector™ contains the highest concentration of cells.



Position #	Position location on BDC
7	Tip
6	Near tip
5	Upper middle
4	Middle
3	Lower middle
2	Near base
1	Base

3. Apply 2µl of Bode PunchPrep™ directly onto the 1.2 mm punch in each well.

Note: Ensure that each 1.2mm punch is fully submerged in Bode PunchPrep™. **DO NOT** cover the plate with strip caps or another sealing material. **DO NOT** use a thermal cycler with a closed lid as this will prevent evaporation.

4. After applying the Bode PunchPrep™ solution to each sample, place the 96 well reaction plate on a heat block preset at a temperature of 70˚C. Keep the reaction plate on the heat block for twenty (20) minutes.

Note: Altering the incubation temperature and/or time may result in poor performance.

5. After the 20 minute incubation, remove the plate from the heat block.

6. Prepare the PCR amplification mix according to the PowerPlex® 16HS Technical Manual.

7. Add 25µl of the PowerPlex® 16HS amplification mix to each well.

8. Seal reaction plate with caps or an appropriate sealing material.

9. Centrifuge the reaction plate for 20 seconds at 3000 rpm.

10. Place the plate on a thermal cycler programmed according to the PowerPlex® 16HS Technical Manual for 27 cycles.

11. When the amplification program has finished, continue processing the samples through the DNA analysis process per the user's specified processes.

c. PowerPlex® 18D

Note: An initial sensitivity experiment must be performed with each desired amplification system to determine the optimal cycle. Cycle recommendations listed below were the optimal cycles determined during internal testing. Results may vary based on individual amplification and detection instruments.

Materials to be Supplied by the User:

- 96 well reaction plate
- 70˚C heat block capable of accepting a 96 well reaction plate
- PowerPlex® 18D System

1. Place 96 well reaction plate under an ionizing fan for 5-10 minutes to eliminate static.

2. Punch one 1.2 mm punch from each Bode Buccal DNA Collector™ being processed into individual wells of the 96 well reaction plate.

Note: Remove the 1.2mm punch from an area close to the tip of the Bode Buccal DNA Collector™. Previous studies have shown that the top third of the Buccal DNA Collector™ contains the highest concentration of cells.

BDC Spatial Distribution

Position #	Position location on BDC
7	Tip
6	Near tip
5	Upper middle
4	Middle
3	Lower middle
2	Near base
1	Base

3. Apply 2µl of Bode PunchPrep™ directly onto the 1.2 mm punch in each well.

Note: Ensure that each 1.2mm punch is fully submerged in Bode PunchPrep™. **DO NOT** cover the plate with strip caps or another sealing material. **DO NOT** use a thermal cycler with a closed lid as this will prevent evaporation.

4. After applying the Bode PunchPrep™ solution to each sample, place the 96 well reaction plate on a heat block preset at a temperature of 70˚C. Keep the reaction plate on the heat block for twenty (20) minutes.

Note: Altering the incubation temperature and/or time may result in poor performance.

5. After the 20 minute incubation, remove the plate from the heat block.

6. Prepare the PCR amplification mix according to the PowerPlex® 18D Technical Manual.

7. Add 25µl of the PowerPlex® 18D amplification mix to each well.

8. Seal reaction plate with caps or an appropriate sealing material.

9. Centrifuge the reaction plate for 20 seconds at 3000 rpm.

10. Place the plate on a thermal cycler programmed according to the PowerPlex® 18D Technical Manual for 28 cycles.

11. When the amplification program has finished, continue processing the samples through the DNA analysis process per the user's specified processes.

d. PowerPlex® 21

Note: An initial sensitivity experiment must be performed with each desired amplification system to determine the optimal cycle. Cycle recommendations listed below were the optimal cycles determined during internal testing. Results may vary based on individual amplification and detection instruments.

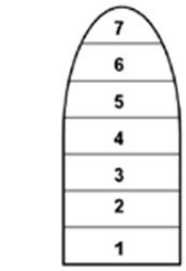
Materials to be Supplied by the User:

- 96 well reaction plate
- 70˚C heat block capable of accepting a 96 well reaction plate
- PowerPlex® 21 System

1. Place 96 well reaction plate under an ionizing fan for 5-10 minutes to eliminate static.

2. Punch one 1.2 mm punch from each Bode Buccal DNA Collector™ being processed into individual wells of the 96 well reaction plate.

Note: Remove the 1.2mm punch from an area close to the tip of the Bode Buccal DNA Collector™. Previous studies have shown that the top third of the Buccal DNA Collector™ contains the highest concentration of cells.



Position #	Position location on BDC
7	Tip
6	Near tip
5	Upper middle
4	Middle
3	Lower middle
2	Near base
1	Base

3. Apply 2µl of Bode PunchPrep™ directly onto the 1.2 mm punch in each well.

Note: Ensure that each 1.2mm punch is fully submerged in Bode PunchPrep™. **DO NOT** cover the plate with strip caps or another sealing material. **DO NOT** use a thermal cycler with a closed lid as this will prevent evaporation.

4. After applying the Bode PunchPrep™ solution to each sample, place the 96 well reaction plate on a heat block preset at a temperature of 70˚C. Keep the reaction plate on the heat block for twenty (20) minutes.

Note: Altering the incubation temperature and/or time may result in poor performance.

5. After the 20 minute incubation, remove the plate from the heat block.

6. Prepare the PCR amplification mix according to the PowerPlex® 21 Technical Manual.

7. Add 25µl of the PowerPlex® 21 amplification mix to each well.

8. Seal reaction plate with caps or an appropriate sealing material.

9. Centrifuge the reaction plate for 20 seconds at 3000 rpm.

10. Place the plate on a thermal cycler programmed according to the PowerPlex® 21 Technical Manual for 26 cycles.

11. When the amplification program has finished, continue processing the samples through the DNA analysis process per the user's specified processes.

4. Troubleshooting

This manual addresses problems specifically related to the use of Bode PunchPrep™ with Bode Buccal DNA Collectors™. For information relating to a problem with a specific amplification kit, please refer to the kit manufacturer's technical manual.

If questions and/or answers are not addressed or provided below, additional information can be obtained online at www.bodetech.com, by calling Customer Service at 703-646-9740, or by email at bode.service@bodetech.com.

Problem	Causes and Solutions
Weak profiles and/or missing alleles	**1.2mm punch did not contain enough DNA.** Cellular deposition will vary between individuals. Ensure that the 1.2mm punch is being removed from the top third of the collector. Removing a punch from as close to the tip as possible is ideal.
	Optimal cycle number not determined. Individual amplification and detection instruments vary. Sensitivity experiment must be performed using samples typically encountered during normal processing.
	Incomplete cell lysis. Ensure that 1.2mm punch is submerged in Bode PunchPrep™ prior to placing on the heat block. Do not shorten the incubation time to less than twenty (20) minutes or change the incubation temperature from 70 ˚C.
	Samples not properly incubated. Do not seal 96 well reaction plate during the 20 minute incubation.
	Improper storage or Bode PunchPrep™. Upon receipt, store Bode PunchPrep™ at 4˚C.
	Inactive Bode PunchPrep™. Each vial is designed for a single use. Do not store and reuse remaining solution.
	Samples amplified utilizing a kit that is not listed in Table 1. Do not use Bode PunchPrep™ with an amplification kit not listed in Table 1. Amplification kits not listed in Table 1 have not been evaluated for direct amplification of 1.2mm punches using Bode PunchPrep™.

5. Related Products

Description	Quantity	Catalog Number
Buccal DNA Sample Collection Kit – English	Case of 50	P09D83-50
Buccal DNA Sample Collection Kit – Spanish	Case of 50	P09D84-50
Buccal DNA Sample Collection Kit – English	Each	P09D83-01
Buccal DNA Sample Collection Kit – Spanish	Each	P09D84-01
Buccal DNA Collectors	Case of 100	P01D28
Buccal DNA Collectors	Case of 200	P01D65
Buccal DNA Transport Pouches with Desiccant	Case of 100	P01D18
Archival Cassettes with Cassette Envelopes	Case of 100	P01D32
Adhesive Seals for Archival Cassettes	Roll of 1000	P01D34
Buccal DNA Archival Trays	Box of 11 Trays	P01D57
Buccal DNA Archival Trays	Case 0f 8 Boxes	P01D56
Archival Tray Collector Covers	Pack of 250	P09D68
Archival Tray Pull Straps	Pack of 125	P09D85

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